



Effect of aqueous and alcoholic extracts of Cocoa plant on *Candida spp*

Weam W Haddid, Fatin Noori Abed Mula Abed

Department of Biology, College of Science, University of Mosul, Iraq

Abstract

Forty-six isolates diagnosed, and the samples were obtained from the fungi unit in the College of Science \ Department of Biology \ University of Mosul, they were re-diagnosed in the laboratory after conducting phenotypic and culture tests. And biochemical, the most important of which is the medium of Chrom Agar Candida Medium (CAC), the results showed that the yeasts that were diagnosed belong to the following types: 41 isolates of *Candida albicans* with a percentage of 89.13%, and 3 isolates belonging to the type *Candida glabrata* with a percentage of 6.52%, and one isolate for each of the two types *Candida tropicalis*, *Candida lusitaniae*, with a rate of 2.17% for each type.

A study of the effect of aqueous and alcoholic extracts of cocoa plant seeds on *Candida spp*. The aqueous extracts of the cocoa plant are superior to the alcoholic extracts in terms of their effect Inhibitory depending on the type of yeast

Keywords: cocoa plant, aqueous and alcoholic extracts *Candida albicans*, *Candida glabrata*.

Introduction

Fungi are a large and diverse group of organisms for which they are being eukaryotic Eukarotic, Spore producing, Heterotrophic, which is single-celled or multi -celled (Naranjo-Ortiz and Gabaldon, 2019) [14], and although there are more than 1.5 million species of fungi on Earth, there are approximately 300 species that are considered disease-causing agents, (Naranjo -Ortiz and Gabaldon, 2019) [14]. (Gnat *et al.*, 2021) [6]. Therefore, a lot of scientific research has been directed towards finding alternatives, and the most important of these alternatives is traditional medicine because it has a huge store of herbs and treatments that can be used as a natural source to obtain antifungal agents from plants and animals after conducting scientific research on them, as plants are considered a repository There are many active compounds, which are compounds of secondary metabolites that are distinguished by their antimicrobial properties in a general form like Volatile oils, Tyrate, tannins, alkaloids, flavones, and others (Sher, 2009) [17]. Cocoa beans are the basic material that is found in the most consumed products in the world, and its composition includes polyphenols, methylxanthines, fats and other compounds. Polyphenols and methylxanthines are known to be responsible for several health benefits, particularly through the prevention of cardiovascular and neurological diseases (Jean-Marie, 2021) [18]. Recent in vitro studies of cocoa compounds have revealed protective actions such as anti-diabetic and anti-obesity activities (Rabadan -Chávez *et al*; Crichton, GE; Elias, *et al.*, 2017) [4].

Materials and methods

Diagnostic tests

Phenotypic characteristics of the colonies and microscopy It was completed Examination of appearance for the colonies developing on SDA solid saproid medium and ps Colony colour, shape, texture, diameter, height and odor (Ellis *et al*, 2007) [5]. and microscopic examination Part of the colony has been taken mediated by the carrier (Inoculation loop) then mixed with a drop of lactophenol blue dye cotton and then The sample was spread on a sterile glass slide and covered with a slide cover (Cover Slide)

Then it was examined by light microscopy at 10X power and 40X to observe the false hyphae and spores Giant.

Biochemical tests

test on chrome agar media cand ida

Growth Test On Chrome Agar Candida (CAC)

It was done by taking part of the yeast colony pure Developing with a 24-hour-old sterile vector on the middle of the SDA Then I planned on the middle of the vineyards of Agar Chrom Agar and the plates were incubated at 37°C M for a period of 24-48 hours. Chromium Acar medium is characterized by being a medium (selective) for the development and isolation of yeast and determining its colonies, as the strains were diagnosed according to the manufacturer's instructions that can determine the *Candida spp*, through the color of the colonies and their external shape, showed yeast *C. albicans* in color green while *C. glabrata* Pale pink to creamy and *C. lusitaniae yeast* are pale pink to purple (Horvath *et al*, 2003) [7].

Preparation of plant extracts

described by Abu Ghadeib, Shtayeh was used (1999) And by weighing 10 gm of vegetable powder, then add It has 100 ml of ethyl alcohol in the case of an alcoholic concentration of 70%, and 100 ml of sterile distilled water in the case of an aqueous extract. It is shaken well to mix the components, then it is placed in a shaker incubator at a temperature of 24 °C for 24 hours to increase the extraction rate, after which it is filtered. The mixture was mediated by pieces of sterile medical gauze, and the filtrate was collected, then placed in sterile test tubes, then in a centrifuge at a speed of 5000 cycles /min for 10minutes after which the filtrate was filtered using filter papers of medium diameter. 0.2 2 µm, then place the extract into dishes with a large surface area until the remaining water is drained

In an oven at a temperature of 40 °C until the water evaporates completely and finally a powder is obtained dry the extract, then the weight of the dry matter is. And stored in the freezer,

taken and placed in opaque glass bottles tightly closed,

Prepare the concentrations used

inhibitory effectiveness of the aqueous and alcoholic extracts of the cocoa plant was tested on *Candida* species diagnosed by diffusion method on Sabourauds Dextrose Agar medium Sabourauds Dextrose Agar (SDA) and incubated at a temperature of 37°C for 24-48 hours, after which the inhibition zone minus the drilling diameter was measured.

Statistical analysis

The ready-made statistical analysis program (SPSS) was used, and the averages were compared using L.S.D. at a probability level of 0.01.

Results and discussion

1-Identification of species of the genus *Candida spp.* Using CAC Medium (Chrom Agar Candida)

The results of the diagnosis using chromium agar medium showed the appearance of colonies in several colors on the chroma agar culture medium, which is a differential medium for the genus *Candida*, where the colony of *C.albicans* yeast appeared in a bright green color, while *C.glabrata* yeast appeared in light pink color, while *C. tropicalis* yeast appeared in blue, colonies of *C.Lusitaniae* yeast appeared in pale pink to purple. These results are consistent with the findings of (Mohammed, 2020 and Al-Hamdani, 2020) [11]. This study also agrees with (Mulet Bayona; *et al.*, 2022, Manzoor, *et al.*, 2018) [13, 12], This study contrasted with (Sangare *et al.*, 2021; Aboutalebian *et al.*, 2021) [19, 3], where they mentioned the dominance of *C. albicans*, then the rest of the species, respectively, Fig. (1)

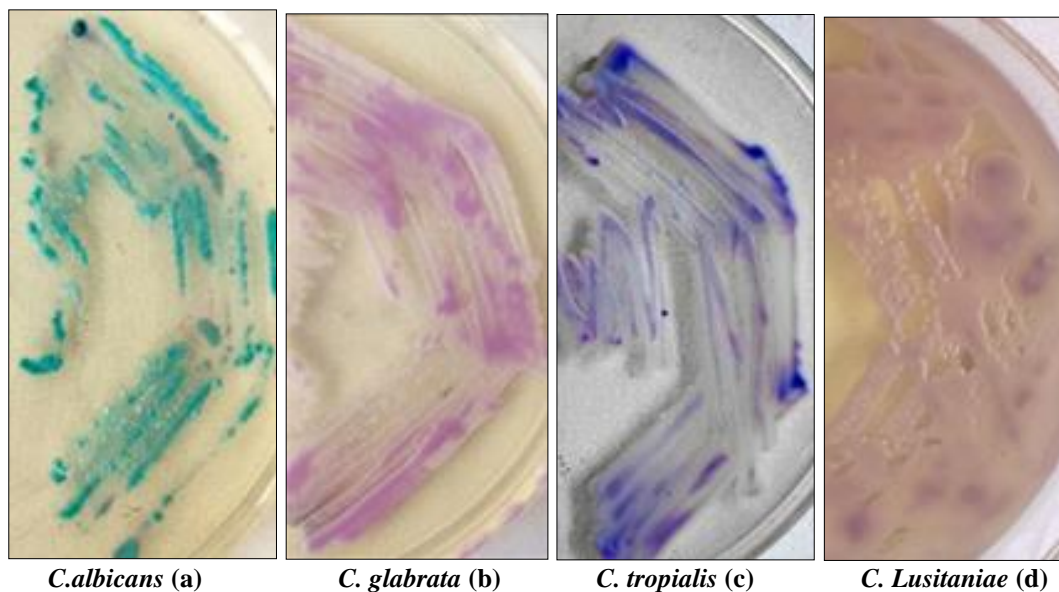


Fig 1: Diagnosis of *Candida spp.* On Chrome Agar (CAC) medium

2. Effect of cocoa extracts on the growth of *Candida* yeasts

The current study showed that there is an inhibitory effect of both the aqueous and alcoholic extracts of the cocoa plant, as they had a broad-spectrum antibody effect on resistant and sensitive *Candida* isolates, and the reason for this is the presence of many bioactive compounds present in cocoa seeds, (Alexandre, *et al.* (2019) [1]

2.1 Effect of cocoa extracts on the growth of *Candida* yeasts

The results showed that the aqueous extract of the cocoa plant gave a good inhibition activity at a concentration of 100% mg/ml. The inhibition rate was 31.00 mm on *C. albicans* yeast, which had the highest inhibition rate, followed by *C. glabrata*1 with an inhibition rate of 20.00 mm, followed by *C. tropicalis*. with an inhibition rate of 19.00 mM at the same concentration, followed by the two yeasts *C.Lusitaniae* and *C.glabrata*3. With the same inhibition rate of 18.00 mm, and they are not significantly different, while the lowest inhibition rate for the same concentration was for yeast *C. glabrata* 2 with an inhibition rate of 17.00, as these

results were identical to the study of (Sultan, Zghair, 2020) [18].

As for the concentration of 80 mg / ml, the highest inhibition rate was for *C. albicans*, where the inhibition rate was 28.00 mm, while the lowest inhibition rate was 10.67 mm for *C. gabrata*2, and the rest of the values were in the middle between these two values.

The concentration of 60 mg/ml of the extract gave less activity than the concentration of 80 mg/ml. The highest inhibition rate on *C.albicans* was 27.00 mm, while the lowest inhibition rate was 13.00 for each of the two yeasts *C.Lusitaniae* and *C.glabrata*3. The rest of the values came between the two mentioned values.

As for the concentration 40 mg/ml, it was less effective than the concentrations 100, 80, and 60 mg/ ml. The highest inhibition rate was 27.00 on *C. albicans*, while the lowest inhibition rate was 10.00 mm on *C. tropicalis*. And the rest of the values were intermediate between the rest of the values.

At a concentration of 20 mg/ml, it gave the highest inhibition rate on *C. albicans*, which was 26.00 mm, while the lowest inhibition rate was 7.33 mm on *C. lusitaniae*, and

the rest of the values were intermediate between the mentioned values.

For a long time, chocolate was only consumed for pleasure, but in the past 20 years, research has shown that dark chocolate and cocoa can have a significant impact on human health due to the high content of polyphenols. Polyphenols Cocoa is rich in polyphenols, such as those found in green tea, and because polyphenols appear to be beneficial for

cardiovascular diseases, cholera, consumption (tuberculosis), scarlet fever, and smallpox.

Previous studies, when phytochemical analysis of medicinal plant extracts, showed the presence of most of the secondary metabolic alkaloids, the free amino group, glycosides, phenols, saponins and tannins (Joshi & Juyal, 2017)^[9]. Also, a previous study showed that medicinal plant extracts had antifungal activity against disease candida (Moshfeghy *et al.* 2018^[10] Table (1), Figure (2).

Table 1: Effect of different concentrations of aqueous extracts of cocoa plant on species *Candida spp.*

yeast effect rate	Effect of concentrations of aqueous extract of cocoa (mg/ml)					types of genus <i>Candida</i>
	%100	%80	%60	%40	%20	
27.8a	31.00	28.00	27.00	27.00	26.00	<i>C. albicans</i>
14.5c	19.00	18.00	17.00	10.00	8.67	<i>C. tropicalis</i>
12.8 f	.00 18	.00 14	13.00	11.67	7.33	<i>C. lusitaniae</i>
15.4b	20.00	19.00	18.00	12.00	8.00	<i>C. glabrata1</i>
12.9 e	17.00	10.67	14.00	13.00	10.00	<i>C. glabrata 2</i>
13.2 d	.00 18	.00 16	13.00	11.33	8.00	<i>C. glabrata 3</i>
	20.5a	17.6b	17c	14.2 d	11.3e	Focus effect rate

Note that the

LSD value of the overlap is (1.3405).

The presence of similar letters indicates that there is no significant difference, but the different letters indicate that there is no significant difference

2. 2 Effect of alcoholic extracts of cocoa plant on *Candida spp.*

The results of our current study showed that the alcoholic extract of the cocoa plant has a low activity compared to the aqueous extract against the studied yeasts, the concentration of 100 mg / ml of the extract showed a higher inhibition activity than the rest of the concentrations, as it reached the highest inhibition rate of 9.00 mm on the yeast *C. albicans*, followed by the yeast *C. tropicalis* with an inhibition rate of 8.00 mm, while the lowest inhibition rate was 0.00 mm on the yeast *C. glabrata1*. between the rest of the values.

As for the concentration of 80 % mg /ml, it had less inhibitory effectiveness, as the highest inhibition rate was 8.00 mm on *C. albicans*, while the lowest inhibition rate was 0.00 mm on *C. glabrata1*, and the rest of the values were mediated.

The concentration was 60% mg/ml, so the highest inhibition

rate was 7.00 on the yeast *C. albicans*, and the lowest inhibition rate was 0.00 mm for each of *C. Lusitaniae.*, *C. glabrata3*, *C. glabrata1*,

As for the concentration of 40 mg / ml, the highest rate of inhibition in this concentration is 6.00 mm, and the least inhibition was for the rest of the yeasts with an inhibition rate of 0.00 mm, and finally the concentration of 20% mg / ml was less inhibition than the rest of the concentrations, so the highest rate of inhibition for this concentration was 5.33 mm on the yeast *C. albicans* The least inhibition rate was 5.00 mm for *C. tropicalis*, and the remaining values were 0.00 mm for other yeasts, Table (2).

The results of the current study showed that the best concentration effect rate for the aqueous extract is 100%, where the concentration effect rate is 20.5%, which is more significant than concentration 80 and 60. and 40 and 20, scoring 17.6, 17 and 14.2 and 11.3, respectively, The current results showed that the best rate of effect of yeast is *C. albicans*; It recorded an average of 27.8 mm It is greater than the effect rate of the yeast *C. Lusitaniae*, *C. glabrata3*, *C. glabrata 2*. And *C. glabrata1* and *C. tropicalis*; As recorded rate r affected _ 14.5, 12.8, and 15.4 and 1.2 and 13.2. There are significant differences between the types of yeasts.

Table 2: the effect of different concentrations of alcoholic extracts of the cocoa plant on Species *Candida spp.*

yeast effect rate	Effect of concentrations of alcoholic extract of cocoa (mg/ml)					types of genus <i>Candida</i>
	%100	%80	%60	%40	%20	
7.06a	9.00	8.00	7.00	6.00	5.33	100% <i>C. albicans</i>
6.66	8.00	7.67	6.67	6.00	5.00	<i>C. tropicalis</i>
1.4	5.00	2.00	0.00	0.00	0.00	<i>C. lusitaniae</i>
0.00	00.0	00.0	0.00	0.00	0.00	<i>C. glabrata1</i>
2.06	4.67	3.67	2.00	0.00	0.00	<i>C. glabrata 2</i>
1.33 e	3.67	3.00	00.0	0.00	0.00	<i>C. glabrata 3</i>
	5.06 a	4.05b	2.61c	2.00 d	1.72 e	Focus effect rate

Note that the LSD value of the overlap is (1.3405). The presence of similar letters indicates that there is no significant difference, but the different letters indicate that there is no significant difference

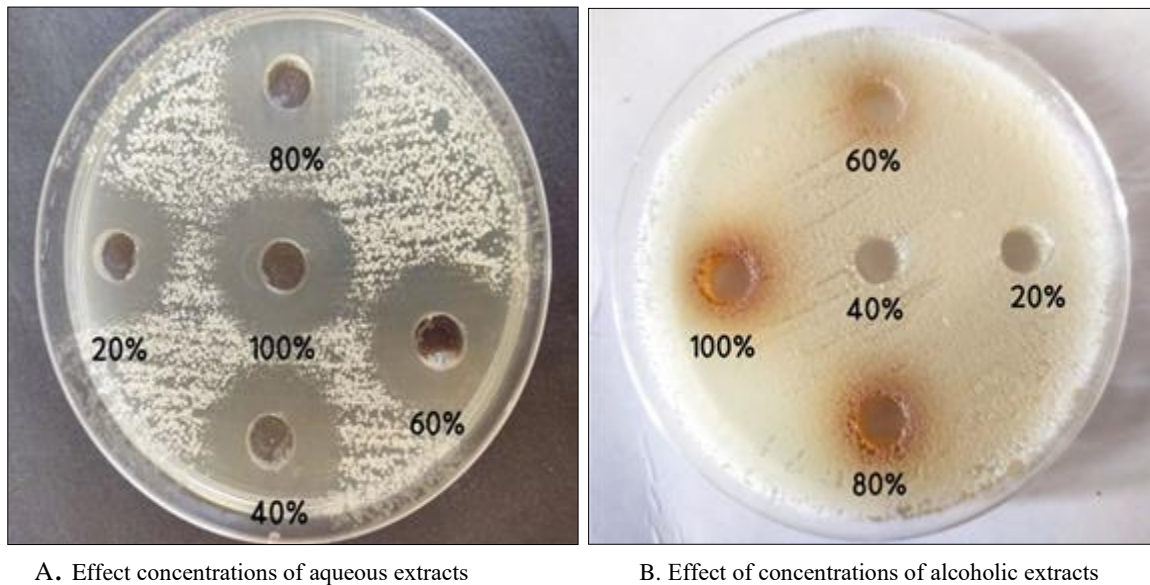


Fig 2: Effect of aqueous and alcoholic extracts of cocoa plants on *Candida* spp.

Conclusion

- The aqueous and alcoholic extracts of cocoa seeds showed a high damping effectiveness in inhibiting *Candida* spp., where the aqueous extracts had a higher inhibitory effect than the extracts. The aqueous extracts had the highest inhibition rate of 20.5 percent, while the inhibition rate of the extracts alcoholic, it was 5.06.
- The aqueous and alcoholic extracts of cocoa beans contain a specific active compound that may cause inhibition of *Candida* spp.

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